

ICP4-induced miR-101 attenuates HSV-1 replication

Xianling Wang^{1¶}, Caifeng Diao^{1¶}, Xi Yang¹, Zhen Yang¹, Min Liu¹, Xin Li¹, Hua Tang^{1*}

1 Tianjin Life Science Research Center and Department of Pathogen Biology, School of Basic Medical Sciences, Tianjin Medical University, 22 Qi-Xiang-Tai Road, Tianjin 300070, China

* Corresponding author

¶These authors contributed equally to this work

Correspondence and requests for materials should be addressed to H.T. (Tel & Fax: +86 22 23542503 E-mail: htang2002@yahoo.com; tangh@tmu.edu.cn)

Supplemental Data

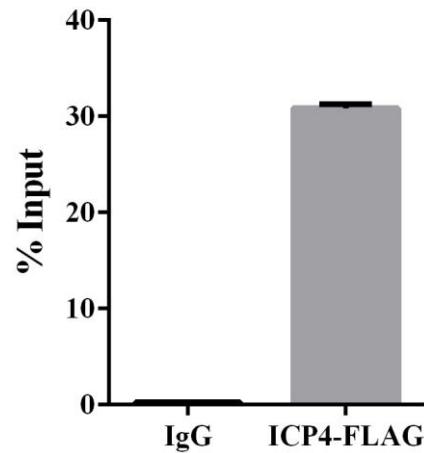


Figure S1. An immunoprecipitation of ICP4 using anti-FLAG and IgG from HeLa cells, which allows for the identification of RCL1/miR-101 promoter fragment. The DNA level of promoter fragment was detected by qPCR.

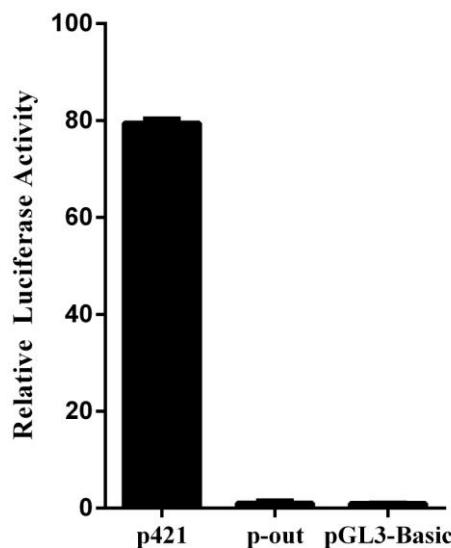


Figure S2. Luciferase activity of RCL1/miR-101 promoter deleted of ICP4 bind site. HeLa cells were transfected with promoter fragments (p421 has the highest promoter activity, p-out stand for the promoter deleted the binding site (59bp), pGL3-Basic as a control pkasmid) for 48h and determined by luciferase activity assays. Data are normalized against the vector

control and error bars present as means \pm SD (n=3).

Figure 3A

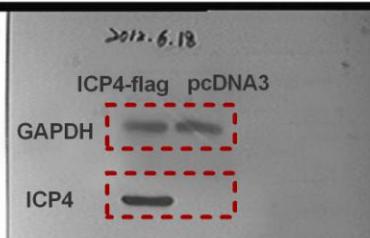


Figure 3E

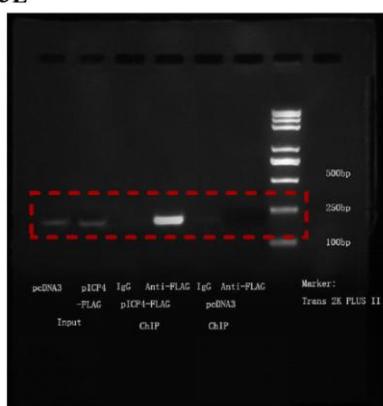


Figure 3G

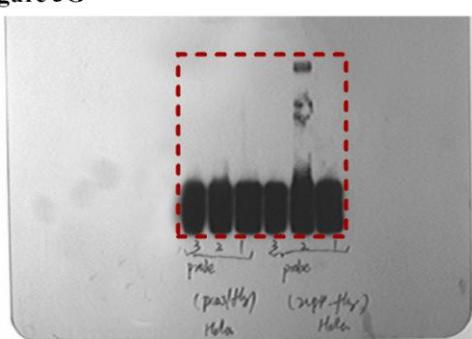


Figure 3B

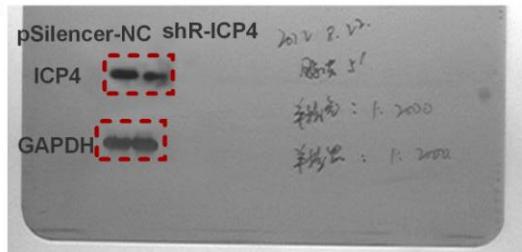


Figure 3F

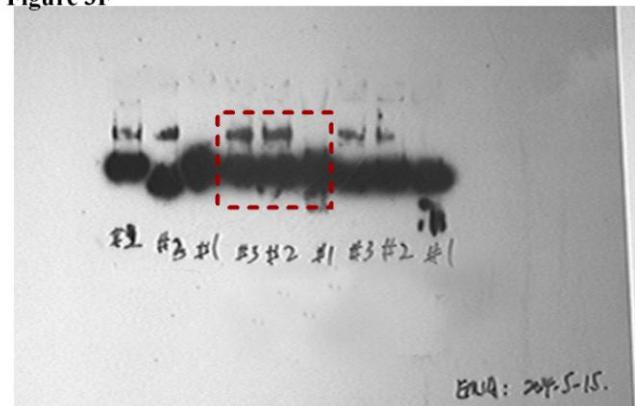


Figure 3H

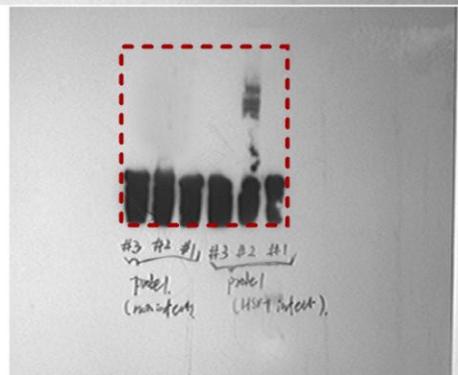


Figure S3. Uncropped, unprocessed images of blots and gels.

Figure 4D

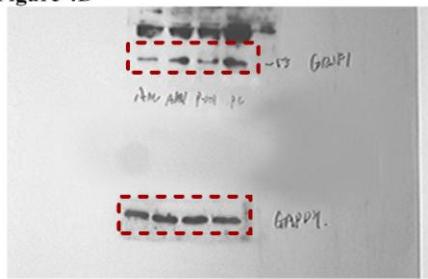


Figure 4F

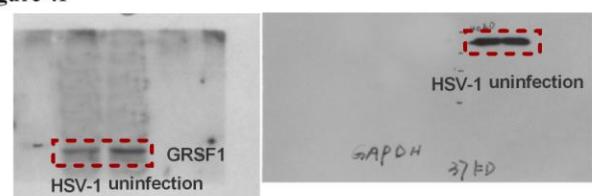


Figure 5A

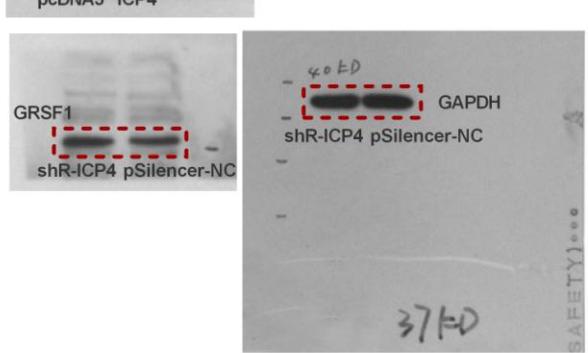
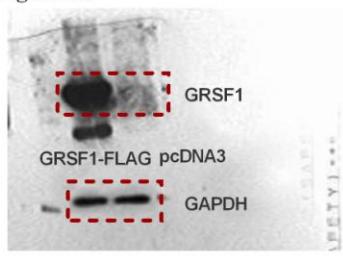


Figure S4. Uncropped, unprocessed images of blots and gels.

Figure 6A

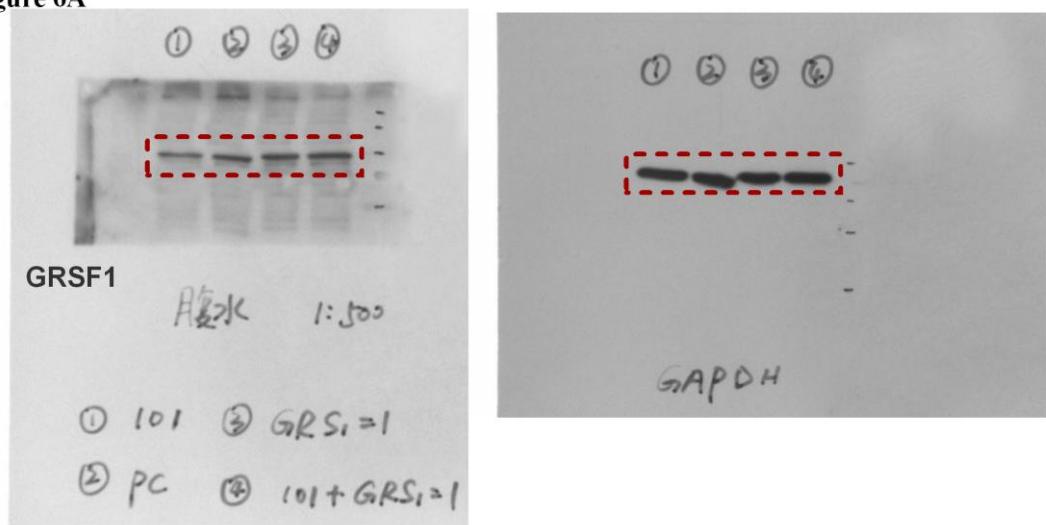


Figure 6D

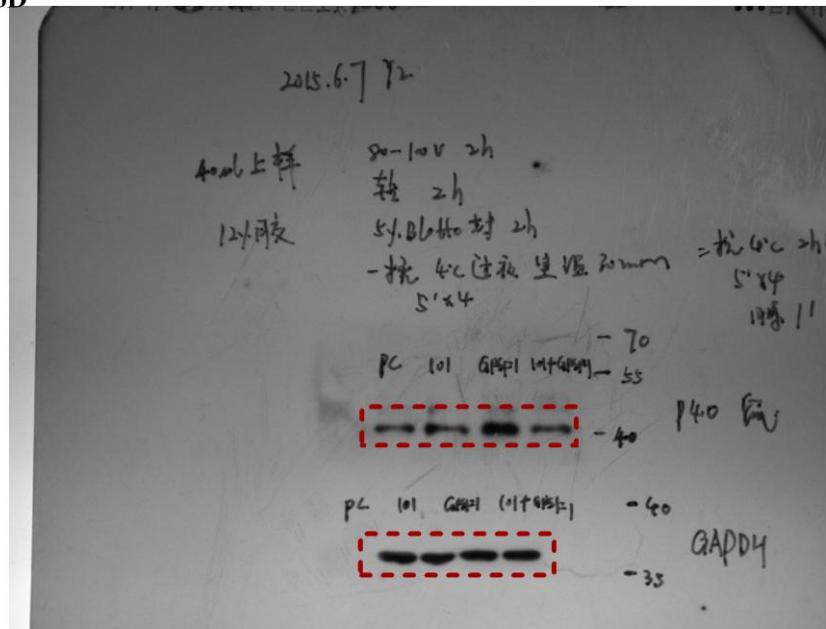


Figure S5. Uncropped, unprocessed images of blots and gels.

Figure5E

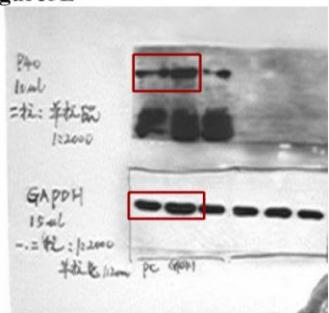


Figure 7A

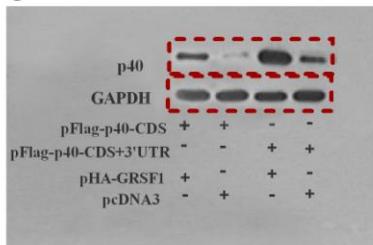


Figure 7C

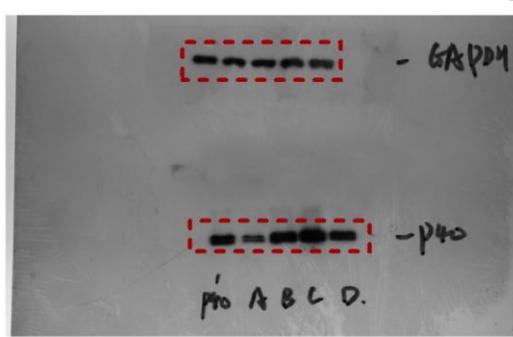


Figure 7B

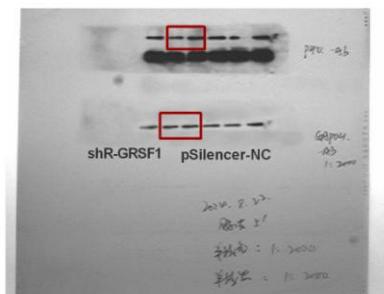


Figure 7E

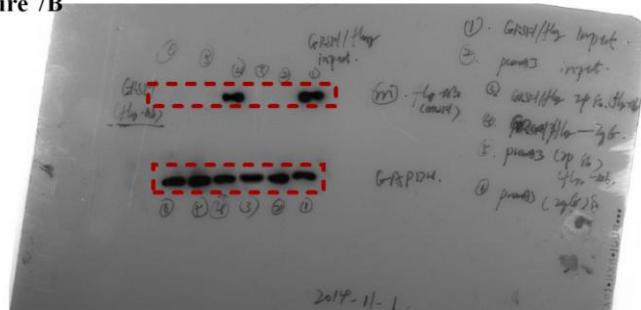


Figure S6. Uncropped, unprocessed images of blots and gels.

Figure 7D

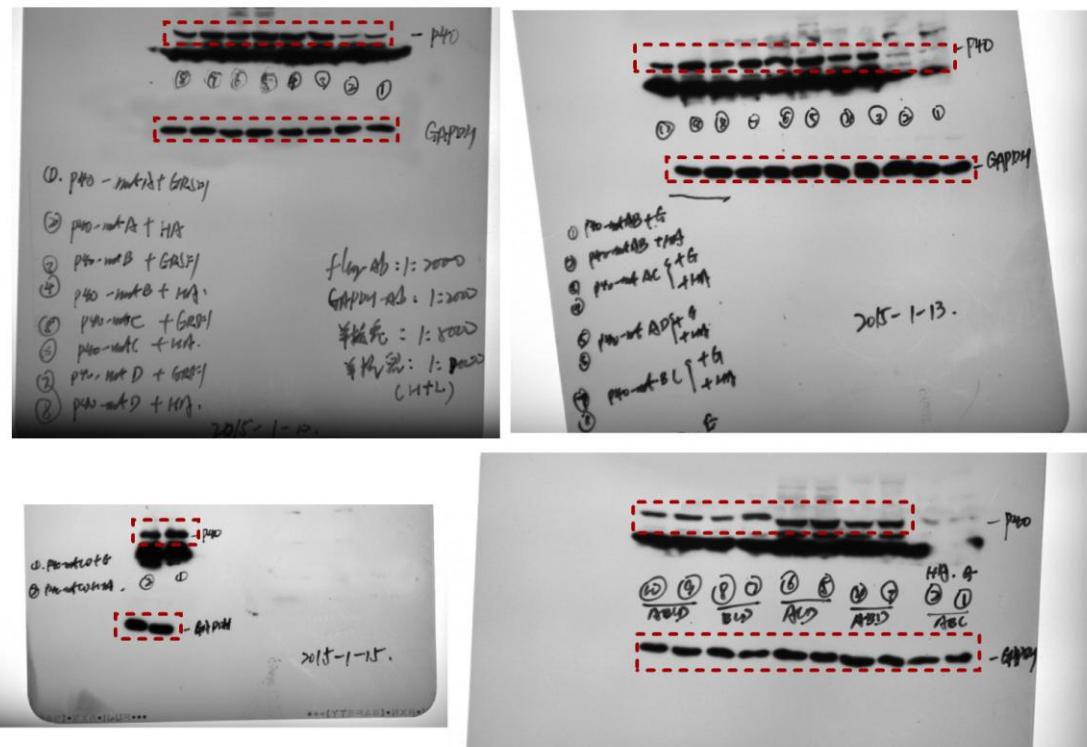


Figure S7. Uncropped, unprocessed images of blots and gels.

Table S1. Primer Sequence in this study.

Primer	Sequence
pGL3-p1060-S	5' GGGGTACCAACTCCCACCGCTTCAG3'
pGL3-p1060-AS	5' GGAATTCGGAGAGACTAGACACCGGGTCAGAG3'
pGL3-p421-S	5' CGCGGTACCTAGCATCTTCACCCAGTAG 3'
pGL3-p421-AS	5' GGAATTCGGAGAGACTAGACACCGGGTCAGAG3'
pGL3-p334-S	5' CGCGGTACCTAGCATCTTCACCCAGTAG3'
pGL3-p334-AS	5' ATAGAATTCTCGGGCCACGCCGCTATTG3'
ICP4-S-ER	5' CGGAATTCATGGCGTCGGAGAACAGCAG3'
ICP4-AS-XI	5' ATAGCCTCGAGTTACAGCACCCGTCCTCTC3'
GRSF1-3'UTR-Top	5' AATTCAAGATGCGAAATCACTGTACTGTAAGCTTC3'
GRSF1-3'UTR-Bot	5' TCGAGAACGCTTACAGTACAGTGATTCGCATCTG3'
GRSF1-3'UTR-Top-mut	5' AATTCAAGATGCGAAATGAGTCTAGACTAAAGCTTC 3'
GRSF1-3'UTR-Bot-mut	5' TCGAGAACGCTTAGTCTAGACTCATTGCGATCTG3'
CRSF1-RI	5' ACGGAATTGAGTCCATGGCCGGCACGCGC3'
CRSF1-XhoI	5' GCAGCACTCGAGGTTTCTTTCATCACCTCGAGGGTGTAGAAGGAGCTGCTGTTTTG3'
shICP4-Top	5' GATCCAACAGCAGCTCCTTCATCACCTCGAGGGTGTAGAAGGAGCTGCTGTTTTG3'
shICP4-Bot	5' AATTCAAAAAAACAGCAGCTCCTTCATCACCTCGAGGGTGTAGAAGGAGCTGCTGTTG3'
shGRSF1-Top	5' GATCCATGAGGATATTCAACCCATGACACTCGAGTGTCTGGGTTGAATATCCTCATTTTTGA3'
shGRSF1-Bot	5' AGCTTCAAAAAATGAGGATATTCAACCCATGACACTCGAGTGTCTGGGTTGAATATCCTCATG3'
miR-101 RT primer	5' TCGTATCCAGTGCAGGGTCCGAGGTGCACTGGATAACGACTCATAACAG3'
pre-miR-101-1-RT	5' TGCCATCCTTCAGTTATC3'

pre-miR-101-2-RT	5' ACCACCATTCTTCAGTTATC3'
U6 RT primer	5' GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAATATGGAAC3'
OligodT primer	TTTTTTTTTTTTTTTT
miR-101-Fwd	5' GCCCGCTCTTGTTATCTAG3'
U6-Fwd	5' TGCAGGTGCTCGCTCGGCAGC3'
Reverse	5' CCAGTCAGGGTCCGAGGT3'
pre-mir-101-1-S	5' TGCCCTGGCTCAGTTATCAC3'
pre-mir-101-1-AS	5' TGCCATCCTTCAGTTATCACAG3'
pre-mir-101-2-S	5' ACTGTCCTTTTCGGTTATCATG3'
pre-mir-101-2-AS:	5' ACCACCATTCTTCAGTTATCACAG3'
RCL1-S	5' GTGAAGAACTCAAGGGTGGG3'
RCL1-AS	5' AAGCAAGTGGCTATTCTGT3'
β-actin-S	5' CGTGACATTAAGGAGAACGCTG3'
β-actin-AS	5' CTAGAACGATTGCGGTGGAC 3'
GRSF1-qPCR--S	5' GTCCTCTGCCCTGAACAGCCTTA3'
GRSF1-qPCR-AS	5' CCAATTCAATGTGAGTGTCTTCG3'
ChIP-S	5' TAGCATCTCACCCCCAGTAG3'
ChIP-AS	5' GCTGCCATTGGTCCGTAGTG3'
3' biotin-probe	5' AGCATCTTCACCCCAGTAGTTGCCGTAAGTAGTAATGCTAAGAATCCGGCACGTCGTAG3'
Unlabeled-probe	5' AGCATCTTCACCCCAGTAGTTGCCGTAAGTAGTAATGCTAAGAATCCGGCACGTCGTAG3'
gD-qPCR-S	5'CGCTTGGTTTCGGATGGGA3'
gD-qPCR-S	5'CTTCACCGAGCCGCAGGTA3'